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September 15, 1992

Commander Peter Kent, MD
Office of Naval Research
Combat Casualty Care Research Area
Naval Medical Research & Development Command
Naval Medical Command, National Capitol Region
Code 405
Bethesda, MD 20814-5044

Subject: Periodic Administrative Report for Award N00014-90-J1797
Liquid Collagen Wound Coverings

Dear Commander Kent:

Attached is a brief summary of research progress since our last report of May 13, 1992.

Yours sincerely,

J. Peter Bentley, PhD
Professor of Biochemistry and Molecular Biology

cc: Administrative Grants Officer
Director, Naval Research Laboratory
Defense Technical Information Center
Office of Chief Of Naval Operations
Bureau of Medicine and Surgery

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Freeze Dried Collagen Preparations

The collaboration with Oregon Freeze Dry, Inc. continues. The following parameters have been successfully concluded.

Sterility

Reducing the viscosity of the collagen preparation by reducing the concentration to 3 mg/ml has allowed filtration through two 0.45 micron membrane filters in tandem and direct dispensing of this filtered material into pre-sterilized flasks with 0.2 micron hydrophobic filter membranes incorporated into the perforated lids. These flasks are supplied by the tissue culture division of Corning, Inc. Collagen solution is poured to a 5-6 mm depth, which is desirable for uniform lyophilization. The material is lyophilized from a concentration of 3 mg/ml, but is re-dissolved at a concentration of 6 mg/ml prior to the addition of iodine and the development of the gel. Following storage we can detect no microorganisms in these preparations.

Shelf Life

Some of the lyophilized collagen already packaged and distributed to the Oregon Burn Center at Emanuel Hospital has been sampled for ease of rehydration after storage at room temperature in a closed tube containing atmospheric air. After a period of 9 months, rehydration occurred within 90 seconds and no particulate matter could be detected following centrifugation. We are currently exploring with Oregon Freeze Dry the possibility of sealing the flasks in plastic-lined foil pouches, purged of atmospheric air, which is then replaced with nitrogen. Periodic sampling to check rehydration quality is also underway.

Vehicle for Growth Factors

As reported on May 13, we have molded collagen crosslinked with DOPA in plastic syringes and incorporated platelet derived growth factor (PDGF) into this material, which was subsequently implanted subcutaneously in rabbits. After being in place 15 days and 45 days the plugs were studied histologically. A much greater cellular infiltrate was noted in the PDGF-treated collagen preparations, suggesting that this crosslinked collagen material can be of use as an implant. We have recently developed a collaboration with Scios, Inc. of Mountainview, California. They have supplied us with quantities of recombinant human fibroblast growth factor (FGF) and with human recombinant heparin binding epidermal growth factor (EGF). We are conducting experiments to determine the rate of release of FGF from DOPA crosslinked collagen. This collagen, molded as before in plastic syringes, together with FGF (containing tracer amounts of I^{125} labeled FGF), was cut into small plugs.

The plugs of collagen were suspended in known volumes of buffer which were sampled periodically for the release of radioactivity. Approximately 25% of the FGF is released from the collagen in a short period of time (one hour). Most of this comes out in the first few minutes, suggesting it is immediately contiguous with the surface. The vast majority of the FGF is not released from the DOPA-collagen material and it is possible that it is either trapped in the interstices of the collagen or is in fact crosslinked by a DOPA mechanism to the collagen. The experiment was repeated and the collagen/FGF plugs were placed in tissue culture wells together with 3T3 fibroblasts. No difference was seen in the rate of growth of these fibroblasts, but we have just been informed that this particular strain of fibroblasts is particularly nonresponsive to FGF and the experiment will now be repeated using dermal derived fibroblasts.

Human Studies

As reported earlier, a collaboration with the Oregon Burn Center at Emanuel Hospital and Medical Center in Portland has been developed and many wound healing kits have been supplied to Phillip Parshley, MD, director of this center. Unfortunately, delays have been experienced in receiving approval from the Emanuel Hospital Human Subjects Committee and use of this material is still pending.